REMARKS

Status of the Claims

Claims 1-21, 23-41 and 125 were under examination. Claims 2-8, 19, 22, 36 and 37 are canceled herein without prejudice or disclaimer. Applicants reserve the right to prosecute the canceled subject matter in one or more divisional or continuation applications. Claims 1, 9-16, 21, 38, 39, 40 and 41 are amended. No new matter has been added by the amendment.

Amendment to the Specification

For convenience, reference to support in the Specification refers to the published application (publication No. 20040219203).

Paragraph [0057] is amended to correct an obvious grammatical error.

Amendment to the Claims

Claim 1 is amended to delete the term "immunoconjugate" and also the phrase "conjugated to one or more lipids, polymeric carriers, micelles, nanoparticles, or combinations thereof." Claim 1 is further amended to include the element "one or more anti-CD74 antibodies or antigen-binding fragments thereof reactive with the epitope of CD74 to which the LL1 antibody binds." Support for this amendment can be found throughout the Specification at least at paragraphs [0016], [0017], [0074], [0075]. Support for the element "wherein the anti-CD74 antibodies or antigen-binding fragments thereof are covalently bound to the PEG component of a PEG-lipid conjugate incorporated into a liposome" can be found throughout the Specification at least at paragraphs [0010] and Example 1. Support for the element "wherein one or more effectors are incorporated into the liposome in unmodified active form, or covalently bound to the lipid component of the liposome, or covalently bound to the PEG component of the liposome" can be found throughout the Specification at least at paragraphs [0007], [0011], [0033], [0096] and [0105]. Support for the element "wherein said anti-CD74 antibodies are chimeric, human or humanized" can be found throughout the Specification at least at paragraphs [0016], [0017], [0018], [0019], [0061]-[0067], [0093], Figures 2, 3 and 4, and original claims 36 and 37.

Claims 9, 10, 13-16 are amended to provide proper antecedent basis in light of the amendments to Claim 1. Claims 38 and 40 are amended to provide proper antecedent basis and to change the dependency from canceled claim 36 to amended claim 1. Claim 38 is also amended to delete the words "the light and heavy chains of the."

Claim 11 is amended to delete reference to certain antigens. Claim 12 is amended to replace the phrase "conjugated to one or more lipids, polymeric carriers, micelles, nanoparticles, or combinations thereof" with "covalently bound to the PEG component of a PEG-lipid conjugate incorporated into a liposome." Support for the amendment can be found at least at Paragraphs [0009], [0010], [0094] and [0095]. Claim 21 is amended to delete the term "hormone." Claim 39 is amended to delete the term "multivalent and multispecific" so that it only recites "multivalent." Claim 41 is amended to correct a grammatical error.

Priority Date

The instant application is a continuation-in-part of USSN 10/314,330, which was a continuation of USSN 09/965,796, which was a continuation of 09/307,816 (filed on May 10, 1999). The Office Action mailed 4/23/07 stated that "The prior applications. . . fail to provide a written description of the instant claims composition comprising *nanoparticles* . . . Accordingly, the instant application is given the effective priority date of June 17, 2003, filing date of 60/478,830 which describes CD74 binding molecules conjugated to nanoparticles." [Office Action of 4/23/07, at page 2. (emphasis added).] In the last response, Applicants traversed this statement and referred to the text of U.S. Patent No. 6,306,393 which issued from 09/307,816, filed May 10, 1999 for written description support for the claimed subject matter. [Response to OA of 4/23/07, at pages 17-18.]

The Action states, on page 2, that the text of U.S. 6,306,393 (which issued from USSN 09/307,816 filed May 10, 1999) regarding the conjugation of anti-CD22 and anti-CD19 antibodies to submicron lipid emulsions "fails to adequately describe the genus of conjugates now claimed which are anti-CD74-*nanoparticle* conjugates. . . . Accordingly, the instant application is given the effective priority date of June 17, 2003, filing date of 60/478,830." [Action at page 2, emphasis added.]

While applicants traverse this assertion, in the interest of advancing the prosecution, applicants have deleted the term "nanoparticles" from claims 1 and 12. Thus, Claim 1 no

longer recites anti-CD74-nanoparticle conjugates. Applicants respectfully submit that the instant claims are entitled to the priority date of USSN 09/307,816, which is May 10, 1999.

Rejection of Claims Under 35 U.S.C. 112, 1st Paragraph, indefiniteness

Claims 38, 39

Claims 38 and 39 were rejected under 35 U.S.C. 112, 2nd paragraph for being indefinite. While applicants traverse with this rejection, in the interest of advancing prosecution and clarifying the claimed subject matter, Applicants have amended claim 38 to delete the phrase "the light and heavy chains of." Applicants submit that claim 38 is no longer indefinite. Since claim 39 depends from claim 38, this claim is also not indefinite. Applicants request withdrawal of this rejection.

Rejection of Claims Under 35 U.S.C. 112, 1st Paragraph, enablement

Claims 11, 12, 21, 32, 33, 39 and 41 were rejected under 35 U.S.C. 112 1st paragraph for lack of enablement.

Claims 11, 12, 21, 39

While Applicants traverse with the enablement rejection, in the interest of advancing prosecution, Applicants have amended claims 11, 12, 21, and 39 as follows. Claim 11 is amended to remove reference to the antigens CD4, CD5, CD8, CD40L, MUC1, MUC2, MUC3, MUC4, tenascin, VEGF, EGFR, CEA, placental growth factor, carbonic anhydrase IX, CSAp and ILGF. Claim 21 is amended to delete the term "hormone." Claim 39 is amended to delete the term "multispecific." Applicants submit that in light of these amendments claims 11, 21 and 39 should be seen as fully enabled. Since claim 12 depends from claim 11, claim 12 should also be seen as fully enabled. Accordingly, Applicants request withdrawal of these rejections.

Claim 41

Applicants traverse this rejection. Claim 41 claims a composition that comprises antibodies or antigen-binding fragments thereof reactive with the epitope of CD74 to which the LL1 antibody binds (see amended Claim 1), which may be a diabody, triabody, or tetrabody. Paragraph [0057] teaches that "[t]he multivalency of the antibody fusion protein means that it can take advantage of multiple interactions in binding to an antigen, thus

increasing the avidity of binding to the antigen. Specificity indicates how many antigens or epitopes an antibody fusion protein is able to bind; i.e., monospecific, bispecific, trispecific, multispecific. Using these definitions, a natural antibody, e.g., an IgG, is bivalent because it has two binding arms but is monospecific because it binds to one epitope. *Monospecific, multivalent fusion proteins have more than one binding site for an epitope but only binds with one epitope, for example a diabody with two binding site reactive with the same antigen*." (emphasis added.) Thus, the antibodies claimed in Claim 41 are multivalent and monospecific, not multispecific. Since the Specification is enabling for monospecific antibodies, Applicants respectfully submit that claim 41, which is directed to a monospecific diabody, triabody or tetrabody, is enabled and request withdrawal of the rejection.

Claims 21, 32, 33

Applicants traverse these rejections. Claim 21 and 32 recite an immunomodulator and Claim 33 recites various examples of immunomodulators. In rejecting these claims the Action states that "the specification has not provided guidance on how to use the required interleukins to treat B cell lymphomas which could potentially stimulate and /or increase the malignant cells. Further regarding the teachings of Hansen ..., one ... would expect that the immunomodulators and interleukins conjugated to the anti-CD74 antibody would be internalized. The Specification has not provided any objective evidence that an immunomodulator or interleukin delivered to the cytosol by an internalized anti-CD74 antibody would exert a therapeutic effect . . ." [Action at page 4].

First, Applicants submit that all the immunomodulators recited in Claim 33 are inhibitory and that none of them are known to stimulate or increase malignant cells.

Second, there is ample evidence in the literature that most immunomodulators, such as interferons and interleukins, when conjugated to antibodies, are able to enhance the therapeutic effects of the antibodies. For exemplary publications please see Gillies et al., Hybridoma, 10(3):347-56 (June 1991); Gillies et al., Proc. Natl. Acad. Sci. U.S.A. 89(4):1428-32 (February 1992); Gillies, U.S. Patent 5,650,150 (patented 07/22/1997); Pelham et al., Cancer Immunology, Immunotherapy 15(3):210-16 (September 1983); Xiang, Human Antibodies 9(1):23-26 (1999). [Abstracts attached.]

Please note that the Gillies publications disclose such effects using an anti-GD2 antibody (conjugated to cytokines such as lymphotoxin, IL-2 or GM-CSF), which were

known to get internalized. See Wargalla and Reisfeld, Proc. Natl. Acad. Sci. U.S.A. 86(13):5146-50 (July 1989) (disclosing the cellular internalization of the anti-GD2 antibody). [Abstract attached.] Furthermore, the evidence in the literature suggested that most antibodies binding to the cell surface do get internalized [See Kyriakos, Cancer Res. 52(4):835-42 (February 1992), (abstract attached)], thus dispelling any concerns regarding the effectivity of immunomodulators delivered to the cytosol by an internalized antibody.

Therefore, a skilled artisan reading the instant application in the light of the general knowledge in the art at the time, would have known that an immunomodulator or interleukin delivered to the cytosol by an internalized anti-CD74 antibody would exert a therapeutic effect and would have been able to practice the claimed invention without undue experimentation. "The test of enablement is whether one reasonably skilled in the art could make or use the invention from the disclosures in the patent coupled with information known in the art without undue experimentation." MPEP 2164.01, citing *United States v. Telectronics, Inc.*, 857 F.2d 778, 785, (Fed. Cir. 1988). Therefore, Applicants respectfully submit that claims 21, 32 and 33 are enabled and request withdrawal of the rejections.

Rejection of Claims Under 35 U.S.C. 103, obviousness

Lundberg rejections

Claims 1-10, 13-21, 27, 35 and 125 were rejected under 35 U.S.C. 103(a) as being unpatentable over Pawlak-Byczkowska (Canc. Res. 1989, 49:4568-77) as evidenced by Juweid (Nuclear Medicine Communications, 1997, Vol. 18, pp 142-148) in view of Lundberg (J. Pharm. Pharmacol. 1999, 51:1099-1105) and Hansen (Biochem. J. 1996, 320:293-300).

The Action acknowledges that "Pawlak-Byczkowska et al do not teach the specific composition comprising a LL1 conjugate and one or more effectors." [Action at page 6]. The Action asserts that Lundberg discloses conjugation of LL2 antibody with a long-circulating drug carrier lipid emulsion. The Action suggests that it would have been prima facie obvious to substitute the LL1 antibody for the LL2 antibody in the composition disclosed by Lundberg. Applicants disagree with this suggestion and argue that given the differences between LL2 and LL1 a skilled artisan would not have had a reasonable expectation of success in doing so. Furthermore, Applicants submit that all elements of the amended claim 1 are not disclosed by the cited prior art.

However, Applicants emphasize the point that Lundberg is not even proper prior art to the instant application, especially in view of the deletion of the term "nanoparticles" from amended claim 1. As noted before, in view of the amended claim 1 the instant application is entitled to the claimed priority date of May 10, 1999. Therefore, Lundberg is not a prior art reference, since Lundberg publication was published in October, 1999.

Neither Pawlak-Byczkowska, nor Juweid nor Hansen, either alone or in combination, disclose all elements of the claimed composition, specifically the elements "the anti-CD74 antibodies or antigen-binding fragments thereof are covalently bound to the PEG component of a PEG-lipid conjugate incorporated into a liposome;" or "one or more effectors are incorporated into the liposome in unmodified active form, or covalently bound to the lipid component of the liposome, or covalently bound to the PEG component of the liposome." Therefore, Applicants respectfully submit that the amended claim 1 is not obvious over the cited prior art and request that this rejection be withdrawn.

Claims 1-10, 13-21, 27, 35-38 and 125 were rejected under 35 U.S.C. 103(a) as unpatentable over Pawlak-Byczkowska, Juweid, Lundberg and Hansen further in view of Schlom (In: Molecular Foundations of Oncology, Samuel Broder, Ed.,1991, pages 95-34); Pawlak-Byczkowska, Juweid, Lundberg and Hansen and further in view of Greenwood (In: Protein engineering of antibody molecules, for therapeutic and prophylactic applications in man, Clark, ed., 1993, pages 89 and 97); and Pawlak-Byczkowska, Juweid, Lundberg and Hansen further in view of Nakagawa (Journal of Neurooncology, 1999, vol. 45, pp 175-183).

As noted above, Lundberg is not proper prior art (and given the significant differences between LL2 and LL1 a skilled artisan would not reasonably expect to substitute LL1 for LL2) and Pawlak-Byczkowska, Juweid or Hansen, either alone or in combination, do not disclose all elements of the claimed composition. Furthermore, neither Schlom, nor Greenwood, nor Nakagawa address the deficiencies discussed above. Specifically none of these references disclose the elements of "the anti-CD74 antibodies or antigen-binding fragments thereof are covalently bound to the PEG component of a PEG-lipid conjugate incorporated into a liposome;" or that "one or more effectors are incorporated into the liposome in unmodified active form, or covalently bound to the lipid component of the liposome, or covalently bound to the PEG component of the liposome." Thus, none of the prior art, either alone or in

combination, disclose all elements of amended claim 1 and a *prima facie* case of obviousness does not exist.

Since all remaining claims depend from claim 1 and include all limitations of claim 1, Applicants submit that these claims are also not obvious and request reconsideration and withdrawal of the rejection.

Rybak rejections

Claims 1, 5, 8, 11, 12, 19, 20, 30, 31, 35-38 and 125 were rejected under 35 U.S.C. 103(a) as being unpatentable over Rybak (U.S. 6,395,276). The Action states that "Rybak teaches an immunotoxin comprising the LL1 antibody, wherein said antibody is attached to a toxin moiety by either conjugation or recombinant means" and that "Rybak specifically teaches the LL1 immunotoxin comprising onconase." The Action asserts that onconase in an RNAse thus fulfilling the specific requirements of an effector which is an enzyme. The Action states that Rybak et al. suggest "possible chemical modifications of the immunotoxins of the invention include derivitization with polyethylene glycol (PEG) to extend half-life in the circulatory system and reduce immunogenicity . . ."

Applicants submit that Rybak does not disclose the claimed composition comprising one or more anti-CD74 antibodies or antigen-binding fragments thereof reactive with the epitope of CD74 to which the LL1 antibody binds, wherein the antibodies or antigen-binding fragments thereof are covalently bound to the PEG component of a PEG-lipid conjugate incorporated into a liposome, and wherein one or more effectors are incorporated into the liposome in unmodified active form, or covalently bound to the lipid component of the liposome, or covalently bound to the PEG component of the liposome. Instead, Rybak discloses an immunotoxin in which the antibody is conjugated to the toxin. Rybak only suggests PEG derivatization of the antibody and the toxin (the protein moieties) to extend time of residence in the circulatory system and reduce immunogenicity. [See Rybak abstract, summary of the invention and column 13, lines 5-12.] Since Rybak does not disclose the covalent binding of antibody to the PEG component of a PEG-lipid conjugate incorporated into a liposome and one or more effectors incorporated into the liposome in unmodified active form, or covalently bound to the lipid component of the liposome, or covalently bound

to the PEG component of the liposome, applicants respectfully submit that the 103(a) rejection over Rybak be withdrawn.

Claims 1, 5, 8, 11, 12, 19, 20, 30, 31, 35-38 and 125 were rejected under 103(a) as being unpatentable over Rybak further in view of Bagshawe (Curr Opin Immunol, 1999, vol. 11, pp. 579-583). The Action states that Bagshawe discloses enzymatic effectors targeted to cancer cells which include a glucoronidase, a carboxypeptidase, a beta-lactamase and a phosphatase. However, Bagshawe also does not disclose all elements of amended claim 1. Therefore, Applicants submit that the claim 1 is not obvious over Rybak and Bagshawe and this rejection also be withdrawn. Since all remaining claims depend from claim 1 and include all limitations of claim 1, these claims are also not obvious and applicants request withdrawal of this rejection.

Hua rejections

Claims 1, 5, 20, 21, 24-29 and 125 were rejected over Hua (Human Pathology, 1998, Vol. 29, pp 1441-1446) in view of Torchilin (Crit Rev Ther Drug Carriers, 1991, Vole. 7, pp 275-308).

Action states that Hua discloses the LN2 antibody to CD74 but does not disclose the chelation of LN2 to polymers carrying radionuclide complexes via hard or soft chelators. Action further states that Torchilin et al disclose the chelation of antibodies to polymers carrying radiolabels.

However, neither reference discloses an anti-CD74 antibody that is reactive with the epitope of CD74 to which the LL1 antibody binds. The LN2 antibody disclosed in Hua differs from the LL1 antibody in many aspects such as different mechanisms of action, different trafficking in the cell and different internalization rates as well as different binding domains and different isotype constructs (both of which affect immune function). Furthermore, neither reference, either alone or in combination, discloses the claimed composition comprising antibodies or antigen-binding fragments covalently bound to the PEG component of a PEG-lipid conjugate incorporated into a liposome, and wherein one or more effectors are incorporated into the liposome in unmodified active form, or covalently bound to the lipid component of the liposome, or covalently bound to the PEG component of the liposome. Therefore, applicants submit that claim 1 is not obvious over Hua. Since all

remaining claims depend from claim 1 and include all limitations of claim 1, these claims are also not obvious over Hua and Torchilin.

Claims 1, 5, 34, 35 were rejected over Hua in view of O'Reilly (U.S. 5,792,845) and Kratz (Crit Rev Drug Carrier Sys, 1999, Vol. 16, pp. 245-288).

Action states that Hua discloses the LN2 antibody to CD74 but does not disclose the chelation of LN2 to polymers carrying angiostatin; O'Reilly discloses that angiostatin inhibits the growth of breast cancer and lung cancer; and Kratz discloses the delivery of drugs via drug-polymer conjugate comprising an acid sensitive linkage which is released in vivo at the tumor site, as well as immunotoxins conjugated to polymers.

However, none of the cited prior art discloses the claimed composition. First, none of the references disclose an anti-CD74 antibody that is reactive with the epitope of CD74 to which the LL1 antibody binds. Second, none of the references disclose the claimed composition comprising antibodies or antigen-binding fragments thereof are covalently bound to the PEG component of a PEG-lipid conjugate incorporated into a liposome, and wherein one or more effectors are incorporated into the liposome in unmodified active form, or covalently bound to the lipid component of the liposome, or covalently bound to the PEG component of the liposome. Kratz discloses drug-polymer conjugates in which the linkage is between the drug and the polymer. [See Kratz abstract, Introduction]. Kratz also discloses immunotoxins in which the linkage is between the antibody and the toxic protein. [See Kratz, at page 273, line 6 from the bottom of the page – page 74, line 11 from the bottom of the page]. Thus, the invention in Kratz is fundamentally different from that claimed here. Since none of the prior art cited discloses all elements of amended claim 1, applicants submit that the claim 1 is not obvious over these references. Since all remaining claims depend from claim 1 and include all limitations of claim 1, applicants submit that these are also not obvious and request withdrawal of all rejections.

Conclusion

For the reasons stated above, Applicants submit that the amended claims are in condition for allowance and request withdrawal of the rejections.

Respectfully submitted,

Dated: February 8, 2008

Rita P. Sanzgiri, Ph.D.
Reg. No. 59,846
303-447-7720

1: Hybridoma. 1991 Jun;10(3):347-56.

Links

Expression of genetically engineered immunoconjugates of lymphotoxin and a chimeric anti-ganglioside GD2 antibody.

Gillies SD, Young D, Lo KM, Foley SF, Reisfeld RA.

Research Department, Abbott Biotech, Needham Heights, Massachusetts 02194.

Human lymphotoxin was genetically conjugated to the constant region of a human gamma 1 immunoglobulin gene at the end of either the second (CH2-LT) or third (CH3-LT) constant region domain. The altered heavy chain constant regions were combined in a plasmid vector together with the variable regions of a mouse anti-ganglioside GD2 antibody 14.18 and the human kappa constant region. The resulting immunoconjugate constructs were expressed in transfected hybridoma cells and tested for both their antibody and lymphotoxin activities. The two constructs were assembled to varying degrees depending on whether the third heavy chain constant region was present. Both forms retained their ability to bind antigen and mediate ADCC but only CH3-LT was able to mediate the lysis of melanoma target cells in the presence of human complement. Lymphotoxin activity, as defined in a cytolytic assay with mouse fibroblasts, was found to increase significantly as a function of heavy chain assembly and to be equivalent to unconjugated lymphotoxin. Neither of the constructs were cytotoxic for antigen-bearing melanoma cells that are normally resistant to lymphotoxin and tumor necrosis factor alpha. Such immunoconjugates may prove useful in targeting cytokines to the site of antigen-bearing cells in vivo. In this case, as a means of eliciting an inflammatory response at the site of a solid tumor.

PMID: 1916847 [PubMed - indexed for MEDLINE]

Journal List > Proc Natl Acad Sci U S A > v.89(4); Feb 15, 1992

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Gillies, S.
Reilly, E.
Lo, K.
Reisfeld, R.

Proc Natl Acad Sci U S A. 1992 February 15; 89(4): 1428–1432.

Antibody-targeted interleukin 2 stimulates T-cell killing of autologous tumor cells.

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Abstract

A genetically engineered fusion protein consisting of a chimeric anti-ganglioside GD2 antibody (ch14.18) and interleukin 2 (IL2) was tested for its ability to enhance the killing of autologous GD2-expressing melanoma target cells by a tumor-infiltrating lymphocyte line (660 TIL). The fusion of IL2 to the carboxyl terminus of the immunoglobulin heavy chain did not reduce IL2 activity as measured in a standard proliferation assay using either mouse or human T-cell lines. Antigen-binding activity was greater than that of the native chimeric antibody. The ability of resting 660 TIL cells to kill their autologous GD2-positive target cells was enhanced if the target cells were first coated with the fusion protein. This stimulation of killing was greater than that of uncoated cells in the presence of equivalent or higher concentrations of free IL2. Such antibody-cytokine fusion proteins may prove useful in targeting the biological effect of IL2 and other cytokines to tumor cells and in this way stimulate their immune destruction.

Full text

Full text is available as a scanned copy of the original print version. Get a printable copy (PDF file)

US005650150A

United States Patent [19]

Gillies

[11] Patent Number: 5,650,150

[45] Date of Patent: Jul. 22, 1997

[54] RECOMBINANT ANTIBODY CYTOKINE FUSION PROTEINS

[76] Inventor: **Stephen D. Gillies**, 245 Leavitt St., Hingham, Mass. 02043

[21] Appl. No.: **281,238**

[22] Filed: **Jul. 27, 1994**

Related U.S. Application Data

[63] Continuation of Ser. No. 788,765, Nov. 7, 1991, abandoned, which is a continuation-in-part of Ser. No. 612,099, Nov. 9, 1990, abandoned.

[51] **Int. Cl.**[6] **A61K 39/395**; A61K 39/40; A61K 45/05; C12P 21/04

[52] **U.S. Cl.** **424/134.1**; 424/133.1; 424/85.1; 435/69.7

[58] **Field of Search** 530/387.1, 387.3, 530/388.3, 387.8; 424/134.1, 133.1, 159.1, 147.1, 155.1; 435/69.7

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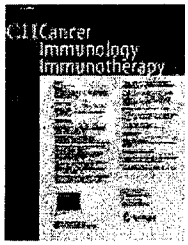
[57] **ABSTRACT**

Immunoconjugates for the selective delivery of a cytokine to a target cell are disclosed. The fusion proteins are comprised of an immunoglobulin heavy chain having a specificity for the target cell, such as a cancer or virus-infected cell, and a cytokine, such as lymphotoxin, tumor necrosis factor alpha, interleukin-2, or granulocyte-macrophage colony stimulating factor, joined via its amino terminal amino acid to the carboxy-terminus of the immunoglobulin. Nucleic acid sequences encoding these fusion proteins and methods of their preparation by genetic engineering techniques are also disclosed.

21 Claims, 9 Drawing Sheets

Journal Article



Interferon-α conjugation to human osteogenic sarcoma monoclonal antibody 791T/36

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Received: 30 March 1983 **Accepted:** 10 May 1983

Summary Human lymphoblastoid interferon-α (IFN-α) has been coupled using N-succinimidyl 3-(2-pyridyldithio) propionate (SPDP) to a murine monoclonal antibody (791T/36) which reacts with antigens expressed on human osteogenic sarcomas. The purified conjugates retain antibody activity as defined by their capacity to compete with binding of fluorescein isothiocyanate-labelled 791T/36 antibody to 791T cells. IFN-α-791T/36 antibody conjugates synthesized with ^{125}I-trace-labelled IFN-α and ^{131}I-trace-labelled antibody also bound to 791T cells, but not to bladder carcinoma T24 cells. The conjugates also retain the capacity of free IFN to activate natural killer cells in human peripheral blood lymphocytes and show specific localization in human osteogenic sarcoma xenografts developing in immunodeprived mice. These findings establish that conjugates containing IFN linked to a monoclonal antibody reacting with osteogenic sarcoma-associated antigens have potential for targeted immunotherapy and in related investigations with antibody has been shown by gamma camera imaging of patients following infusion of ^{131}I-labelled antibody to localize in primary osteogenic sarcomas.

Article

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Human Antibodies
Issue: Volume 9, Number 1 / 1999
Pages: 23 - 36
URL: Linking Options

Targeting cytokines to tumors to induce active antitumor immune responses by recombinant fusion proteins

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Abstract:

Cytokines such as interleukin-2(IL-2), gamma interferon (IFN-\gamma) and alpha tumor necrosis factor (TNF-\alpha) are important mediators in immune responses against tumors. However, their therapeutic efficacy and clinical utilities in treatment of human malignancies are in large part limited due to the low concentrations of cytokine in tumors and the severe toxic side-effects derived from high-dose administration of cytokines. One critical issue to improve therapeutic efficacy is how to increase the local concentration of cytokine in tumors without causing severe side-effects. A series of recent reports demonstrated that the introduction of cytokine genes into tumor cells and subsequent local secretion can circumvent the limitations associated with the systemic cytokine administration. An alternative means of cytokine delivery is to target cytokines to tumor cells with tumor specific antibodies. Thereby, effective local cytokine concentrations can be achieved at the tumor sites without resorting to patient-specific therapy. With the advance in biotechnology, two structurally disparate domains of immunoglobulin and cytokine can be brought together into one fusion protein molecule by protein engineering These engineered antibody-cytokine fusion proteins combine ihe unique targeting ability of tumor-specific antibodies with the multifunctional activity of cytokines. In general, there are two commonly engineered fusion proteins, the F(ab')_2/cytokine expressed in mammalian cells and the single-chain FV/cytokine expressed in Escherichia coli. Both the tumor-binding reactivity and the functional cytokine activity are maintained in most of fusion proteins. Therefore, these fusion proteins may be useful in targeting cytokine to tumors to stimulate immune destruction of tumors, while limiting severe toxic side-effects by the high dose of cytokine administration. Recent preclinical studies have shown that these fusion proteins are able to target cytokines to tumors expressing the tumor-associated antigen in vivo, and to inhibit both

the primary and metastatic tumors in an immune competent animal model. Therefore. these recombinant fusion proteins may represent a new generation of novel immunotherapeutic reagents for the treatment of human malignant diseases.

Keywords:

cytokines, tumor-specific antibodies, genetic engineering, recombinant fusion proteins, antitumor immune mechanisms, animal models

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References:

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Wargalla, U.
Reisfeld, R.

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Rate of internalization of an immunotoxin correlates with cytotoxic activity against human tumor cells.

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Abstract

The relationship between the cellular internalization of an anti-ganglioside GD2 monoclonal antibody (14.G2a) and the toxic effect of its ricin A-chain immunotoxin (14.G2a-RA) was examined on GD2-bearing M21 human melanoma and T293 small cell lung carcinoma cell lines. The capacity for ligand uptake was determined by examining the parameters that contribute to this constant, including the number of cell-surface binding sites and the internalization rate constant (ke). The maximum uptake of 14.G2a is 11-fold greater for M21 than for T293 cells, due to a 2.7-fold difference in binding sites and a 4-fold difference in the rate of antibody internalization. The capacity for ligand uptake correlates with the cytotoxic activity of the 14.G2a-RA immunotoxin against these two cell lines. Furthermore, we were able to demonstrate that the consequence of internalization of 14.G2a-RA is the intracellular release of undegraded ricin A-chain from the antibody. These studies indicate that the rate of internalization is a quantitative parameter that plays a key role in predicting the cytotoxic potency of this immunotoxin.

Full text

1: Cancer Res. 1992 Feb 15;52(4):835-42.

The fate of antibodies bound to the surface of tumor cells in vitro.

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The fate of monoclonal antibodies binding to the surface of human tumor cells in vitro was investigated. Seven antibodies, labeled with 125I, were tested on four cell lines, which included a melanoma and carcinomas of the ovary, kidney, and lung. The antibodies were selected only by the criterion that they not be rapidly internalized via coated pits, so that they would be representative of most antibodies reacting with cell surface antigens. After allowing binding during a 2-h incubation, unbound antibody was removed, and the release of intact or degraded antibody in the supernatant was monitored. The data demonstrate that most bound antibody was gradually degraded and released from the cell over a 2-3-day period, probably via internalization, while only a small fraction, less than 20% for most antibodies, appeared to dissociate intact. One exceptional antibody, MW207, dissociated largely intact. The release of intact antibody was virtually complete within 4 h, and radioactivity released after this time was predominantly in degraded form. These results demonstrate that antibody binding to the surface of viable cells must in general be considered irreversible, and hence the concept of affinity is not applicable. Since an Fab fragment of one of the antibodies dissociated rapidly, such irreversible binding appears to require bivalent attachment. Another conclusion of this study is that most antibodies binding to the cell surface are gradually internalized, which we suggest is due to the normal turnover of cell surface constituents via non-clathrin-dependent endocytosis. Several experimental approaches indicated that a large fraction of antibody retained by the cells, for at least 2 days after binding, was present at the cell surface.

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